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Influence of Drug Absorption, Distribution, Metabolism, and Excretion (ADME) Variants on Sirolimus Blood Levels in Patients Following Allogeneic Hematopoietic Stem Cell Transplantation

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Allelic variants implicated in drug absorption, distribution, metabolism, and excretion (ADME) affect drug pharmacokinetic variability and have been increasingly recognized as important factors in medical therapy. Our recently published study found an association of thrombotic microangiopathy (TMA) with high sirolimus serum levels in a cohort of 177 patients who received sirolimus and tacrolimus as GVHD prophylaxis following allogeneic transplantation. By multivariable analysis, increased risk of TMA was associated with day 14 serum sirolimus levels, prior aGVHD, and myeloablative conditioning. In the current study we explore the possible influence of ADME variants on sirolimus levels and development of TMA in the same patient cohort.

We obtained archived DNA samples from the patients on our TMA study and analyzed them using the iPLEX ADME PGx panel and the MassARRAY® Compact Analyzer. This panel is based on the PharmADME Working Group list and covers >99% of the Pharma ADME Core list; it interrogates 188 mutations and 12 copy number variation assays (in 36 pharmacogenetically relevant genes). Sirolimus levels were measured at least weekly until day 100 with dose adjustments made for target levels and/or clinical toxicity. Possible associations between early sirolimus serum levels (day +14) and assays were evaluated by the Kruskal-Wallis (non-parametric) test and the false discovery rate was used to control for multiple comparisons.

Using this Panel, 179 samples were genotyped, of which 173 showed high quality data. The average call rate for these samples was 98.85% over 200 assays, with a median call rate of 100%. Of these assays, 66 variants were identified that may be of relevance to sirolimus metabolism; other assays were excluded due to homzygosity or >10 % missing data. Using this panel, we found 3 assays showing an association with sirolimus drug level, rs1057910 (CYP2C9*3) at $P = .04$, rs1799931 (NAT2*7) at $P = .03$, and rs2032582 (ABCB1 2677G>A/T) at $P = .007$. In addition, the 0-copy haplotype of UGT2B17 also showed higher levels of sirolimus ($P = .02$). These assays were also tested for an association with TMA, showing a trend for increased TMA in the rs1799931 (NAT2*7) ($P = .08$). However, after adjustment for multiple testing, only rs2032582 maintained statistical significance due to the small sample size.

In conclusion, this pilot study of the iPLEX ADME PGx panel showed feasiblity and provided high quality data. Despite the limitation of small sample size, several genetic variants were implicated in sirolimus levels and may warrant further investigation. Future analysis should focus on specific gene clusters or pathways and will require a large cohort to power validation and training sets.

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CD38 Bright Effector Memory CD8+ T Cell Populations Predict Acute Graft Versus Host Disease

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Introduction: Acute graft versus host disease (aGVHD) is mediated by allogeneic T cell responses. We hypothesized that peripheral blood expansion of activated effector memory T cell populations (TEM) following allogeneic hematopoietic cell transplantation (HCT) would serve as a useful predictor of aGVHD.

Methods: T cells were characterized in peripheral blood samples from 16 consecutive pediatric allogeneic hematopoietic cell transplant (HCT) recipients. Samples were collected prior to transplant and weekly following HCT until day +42. Samples were incubated with fluorochrome-conjugated monoclonal antibodies directed against CD3, CD8, CD38, CD45RA and CCR7, followed by red cell lysis and fixation. Samples were analyzed by flow cytometry on a FACSCanto ll flow cytometer (BD Biosciences). Data was analyzed using FCS Express (De Novo Software). TEM were defined as CD3+ lymphocytes which lacked expression of CD45RA and CCR7. CD38 was used as a marker of activation.

Results: Patients had less than 65% of CD8+ CD38 bright TEMs prior to transplant except for 1 patient with HLH who had 99.8%, and was excluded from analysis. Patients who developed aGVHD (n=5), engraftment syndrome (n=2), or neither (n=8) were observed to develop median maximum expansions of CD8+ CD38bright TEM prior to or on the day of diagnosis of aGVHD, or before day +42, of 49 cells/mcl (range 21-87), 5 cells/mcL (range 0.3-34) and 98cells/mcL (range 0.04-197), respectively ($P = .02$, GVHD versus neither GVHD nor engraftment syndrome). We observed that an absolute number of CD8+ CD38 bright TEM greater than 20cells/mcL was predictive of aGVHD with sensitivity of 100%, specificity of 80%, and a negative predictive value of 100%. The cumulative incidence of aGVHD in patients with greater than 20 CD8+ CD38 bright TEMs/mcL was 71%, and in patients with less than 20cells/mcL, 0% ($P < .01$).

Conclusion: Quantification of peripheral blood CD8+ CD38 bright TEM is a novel predictor of aGVHD.

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Novel Strategy for Non-Invasive Sampling of Epidermal Cytokines Using a Skin Sampling Disc in Acute Skin Graft Versus Host Disease and Engraftment Syndrome

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Novel Strategy for Non-invasive Sampling of Epidermal Cytokines Using a Skin Sampling Disc in Acute Skin Graft versus Host Disease and Engraftment syndrome.

Introduction: The local cytokine milieu of target organs is poorly described in acute graft versus host disease (aGVHD) or engraftment syndrome (ES). A novel and noninvasive methodology using D-Squame (Cuderm Dallas, TX) skin sampling discs was utilized to collect the superficial layer of the stratum corneum in patients with acute skin GVHD or ES and cytokines were measured from the keratinocytes which adhered to the disc.

Methods: D-squame skin sampling discs were placed on the forearm/wrist of 13 pediatric patients (ages 0.25 years-14 years) for 2 minutes and removed. In patients with acute skin GVHD or ES, samples were obtained from the involved area of the forearm/wrist. This procedure was noted to be painless and safe even for young infants. Superficial keratinocytes were sampled by the fully cured medical grade polyacrylate ester adhesive on the disc. Cytokines were extracted from the sampling discs using a buffer made of phosphate buffered saline and 0.25 molar saline and analyzed using a 38 plex human cytokine Milliplex MAP magnetic bead panel (EMD Millipore Corp, Billerica, MA). Patients were divided into 4 groups; acute skin GVHD (n=5), isolated engraftment syndrome (n=2), no acute GVHD or ES (n=2) and healthy pediatric controls (n=4).

Results: IL1a was elevated in patients with aGVHD as compared to healthy controls and patients who did not develop aGVHD or ES ($P = .02$ and $P = .04$) Similarly IL1a was elevated in ES as compared to patients who did not develop aGVHD/ES or to healthy pediatric controls ($P = .05$ and $P = .01$).

Conclusions: These preliminary results demonstrate a novel methodology to measure inflammatory cytokines locally in acute skin GVHD and ES. With further patients and analysis, new approaches to management may be indicated in the future.

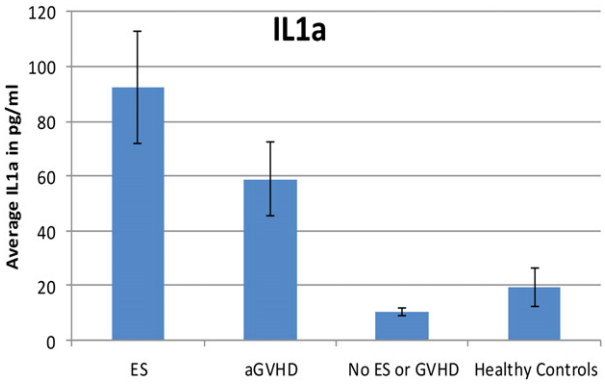

Figure 1. Average values of IL1a measured from the epidermis in patients with ES, aGVHD, no ES or aGVHD and healthy pediatric controls.

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Preliminary Results From a Single Institutional Prospective Study of Alemtuzumab for the Treatment of Steroid Refractory Acute Graft Versus Host Disease in Pediatrics

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Introduction: No clear second line agent exists for the treatment of steroid refractory acute graft versus host disease (SR-aGVHD) in children. Alemtuzumab is associated with partial or complete responses in 50-94% of adult patients. Studies are lacking in pediatric patients. The optimal dose for young patients is unknown. Here we present the first phase of a single center dose-escalation study of alemtuzumab for treatment of acute SR-aGVHD in pediatric patients.

Methods: Patients were prospectively enrolled at the time of diagnosis of grades II-IV acute GVHD. Patients were initially treated with 4mg/kg/day methylprednisolone. If patients failed to improve within 5 days, or worsened within 48 hours, SR-aGVHD was diagnosed and alemtuzumab was administered at 0.2 mg/kg/day for 5 consecutive days (maximum cumulative dose of 31mg). Additional 0.2mg/kg doses were given on days 15, 22 and 29 (maximum dose 10mg/dose). Patients were monitored weekly for response. Partial response (PR) was defined as improvement in stage of GVHD before 28 days following first alemtuzumab administration, and complete response (CR) was defined as achievement of grade zero GVHD by 28 days following the first alemtuzumab administration.

Results: Six patients have been enrolled and 5 patients with grades III (n=4) or IV (n=1) GVHD were treated with alemtuzumab so far. Median age was 12 years (range 1.9-27 years). Organs involved included skin (n=4), gastrointestinal tract (n=4) and liver (n=2) (Table I). Forty percent of patients (n=2) had a CR. Sixty percent of patients (n=3) had a PR. Adverse effects included transient neutropenia or thrombocytopenia (n=3), fever (n=2), or asymptomatic EBV viremia (n=2) and/or adenovirus viremia (n=2).

Conclusion: Alemtuzumab is an active agent for the treatment of SR-a GVHD in pediatric patients. Further dose escalation may increase complete response rates and establish an optimal regimen for successful therapy.

Table I
Response to alemtuzumab

Patient	Organ(s) Involved	Grade at Alemtuzumab Administration	Grade at Day 28	Stage at Alemtuzumab Administration	Stage at day 6	Stage at day 10	Stage at day 14	Stage at day 21	Stage at day 28
1	Skin	III	0	III	I	0	0	0	0
	GI			III	III	0	0	0	0
2	Skin	III	III	I	0	0	0	0	0
	GI			IV	III	III	III	I	II
	Liver			II	II	II	II	II	II
3	Skin	III	III	II	I	0	End of study therapy		
	GI			III	III	III			
4	Skin	IV	IV	II	I	0	End of study therapy		
	Liver			IV	IV	IV			
5	GI	III	0	IV	I	I	I	0	0